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Note

Evidence for intermolecular binding between deacetylated acetan and the glucomannan konjac mannan

Mike J. Ridout, Paul Cairns, Geoffrey J. Brownsey, Victor J. Morris *

Department of Food Biophysics, Institute of Food Research, Norwich Research Park, Colney, Norwich, UK, NR4 7UA

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Abstract

Binary mixtures of deacetylated acetan and konjac mannan form thermoreversible gels under conditions for which the individual components do not gel. Such synergistic behaviour is normally attributed to intermolecular binding between the two polysaccharides. X-ray diffraction data obtained from oriented fibres prepared from deacetylated acetan-konjac mannan gels provides direct evidence for intermolecular binding between the two polysaccharides. The novel heterotypic junction zones appear to be six-fold helices with a pitch of 5.6 ± 0.1 nm. © 1998 Published by Elsevier Science Ltd. All rights reserved

Keywords: Acetan; Konjac mannan; Binary gels; Synergism

The technique of X-ray diffraction of fibres prepared from oriented gels has become the standard method for producing molecular models for the polysaccharide-polysaccharide association within the junction zones of gels [1]. Despite the fact that the preparation of the fibres requires that the gels are stretched, and partially dehydrated, accumulated studies of single polysaccharide systems have shown that, in all but one case, the method provides reliable models for the ordered polysaccharide structure present in the hydrated state [1]. This technique has been extended to study binary polysaccharide gels in order to investigate proposed intermolecular binding between different polysaccharides [2–9]. Such studies have delivered evidence for intermolecular binding between xanthan and galactomannans (xanthan–carob and xanthan–tara) and between xanthan and the glucomannan konjac mannan.

The chemical structure of xanthan (Fig. 1) consists of a cellulose backbone substituted through O-3 on alternate glucose residues with a charged trisaccharide sidechain [10–12]. The sidechains are considered to alter the normal backbone geometry, leading to the formation of a helical structure with five-fold symmetry [13,14]. The polysaccharide acetan, secreted by the bacterium *Acetobacter xylinum* [15], has a similar chemical structure (Fig. 1) to xanthan. Acetan has a cellulose backbone substituted through O-3 on alternate glucose residues with a charged pentasaccharide sidechain [15,16]. The polysaccharides differ in the length

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* Corresponding author. Fax: +44 1603 507723

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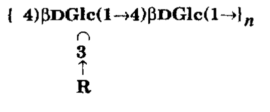
and composition of the sidechain and the type and position of their non-carbohydrate substituents [16–18]. Analysis of the X-ray diffraction patterns obtained from oriented acetan fibres [15,19,20] suggest that acetan adopts a five-fold helical structure similar to that of xanthan. There is growing evidence from a number of studies [18,19,21] that acetan undergoes a thermoreversible conformational (helix-coil) transition in aqueous solution. Thus, given the similarities between xanthan and acetan, it might be expected that acetan would show synergistic interactions with galactomannans and glucomannans. Published studies [21–23] suggest that such interactions do occur for deacetylated acetan–konjac mannan mixtures. This article provides direct evidence from X-ray diffraction studies for intermolecular binding between the two polysaccharides in synergistic gels formed from mixtures of deacetylated acetan and konjac mannan

1. Materials and methods

Acetan was prepared by batch fermentation of *A. xylinum* using the methodology described by MacCormick and coworkers [24], isolated from clarified broths by alcohol precipitation in the presence of 1% KCl, purified by CTAB precipitation [25] and then freeze dried. Complete deacetylation was achieved by alkali treatment using conditions described elsewhere [18]. The structure of the native and deacylated acetan were checked by high resolution nmr. These studies also demonstrated the absence of detectable levels of contaminating protein or nucleic acid impurities. Samples of konjac mannan were purchased from Senn Chemicals AG (Dielsdorf, Switzerland). Solutions of both polysaccharides were prepared by heating aqueous dispersions to 90 °C in sealed tubes. The konjac mannan preparations were centrifuged (4500 g for 1.5 h) to remove insoluble matter. The concentration of the supernatant was determined by evaporation of a small fraction of the sample to dry weight. The acetan and konjac mannan stock solutions were used to prepare mixtures: the solutions were heated to 90 °C in sealed tubes, poured into circular moulds (5 cm diameter), covered, cooled rapidly to room temperature and stored overnight. Rheological measurements were made using an INSTRON 3250 mechanical spectrometer. The gels were measured in the plastic moulds after gluing the moulds to the lower platen.



$$[\,4)\underset{\substack{\frown\\3\\\uparrow\\\text{R}}}{\beta\text{DGlc}}(1\rightarrow 4)\beta\text{DGlc}(1\rightarrow]_n$$

(a) R = βDMan(1→4)βDGlcA(1→2)αDMan(1

(b) R = αLRha(1→6)βDGlc(1→6)αDGlc(1→4)βDGlcA(1→2)αDMan(1

Fig. 1. The chemical repeat units of (a) xanthan and (b) acetan. Xanthan is partially 4,6 substituted with pyruvate or partially O-6 substituted with acetate on the terminal mannose residue, and partially O-6 substituted with acetate on the inner mannose residue. Acetan is partially O-6 substituted with acetate on the mannose and the branched glucose residues.

X-ray diffraction studies have been used to examine whether gelation results from intermolecular binding between the deacetylated acetan and konjac mannan. Hot (90 °C) samples of deacetylated acetan, konjac mannan and mixtures

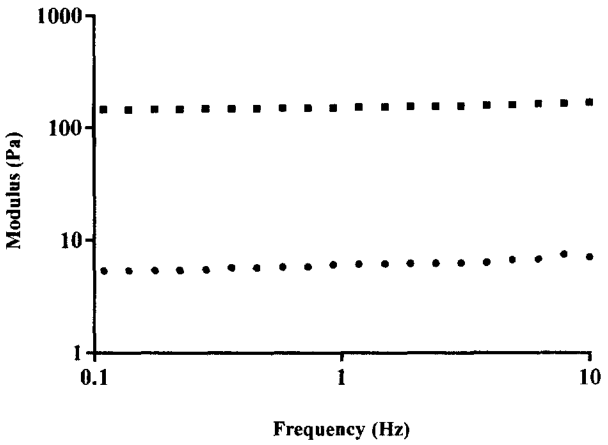


Fig. 2. Mechanical spectrum for a 0.5% w/v 6:4 deacetylated acetan–konjac mannan gel. G′-storage modulus (■) and G″-loss modulus (●). Strain 0.1.

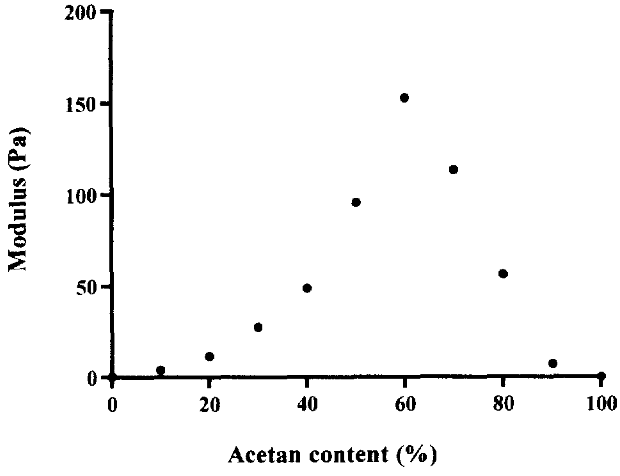


Fig. 3. Variation of G′ with composition for 0.5% deacetylated acetan-konjac mannan gels. Data recorded at a frequency of 1 Hz and a strain of 0.1.

were poured onto PTFE sheets, allowed to cool to room temperature and partially dried to form thin films. These films or gels (formed from gelling samples) were cut into strips, stretched under controlled humidity and allowed to dry. X-ray diffraction patterns were recorded photographically using a flat plate camera, flushed with helium to reduce air scatter, and maintained at a relative humidity of 98%.

2. Results and discussion

The present studies are chiefly concerned with providing evidence for intermolecular binding between deacetylated acetan and konjac mannan. The preparation of fibres for X-ray diffraction requires the partial drying of films. To avoid crystallisation of excess salt the films were prepared from aqueous mixtures of the two polysaccharides.

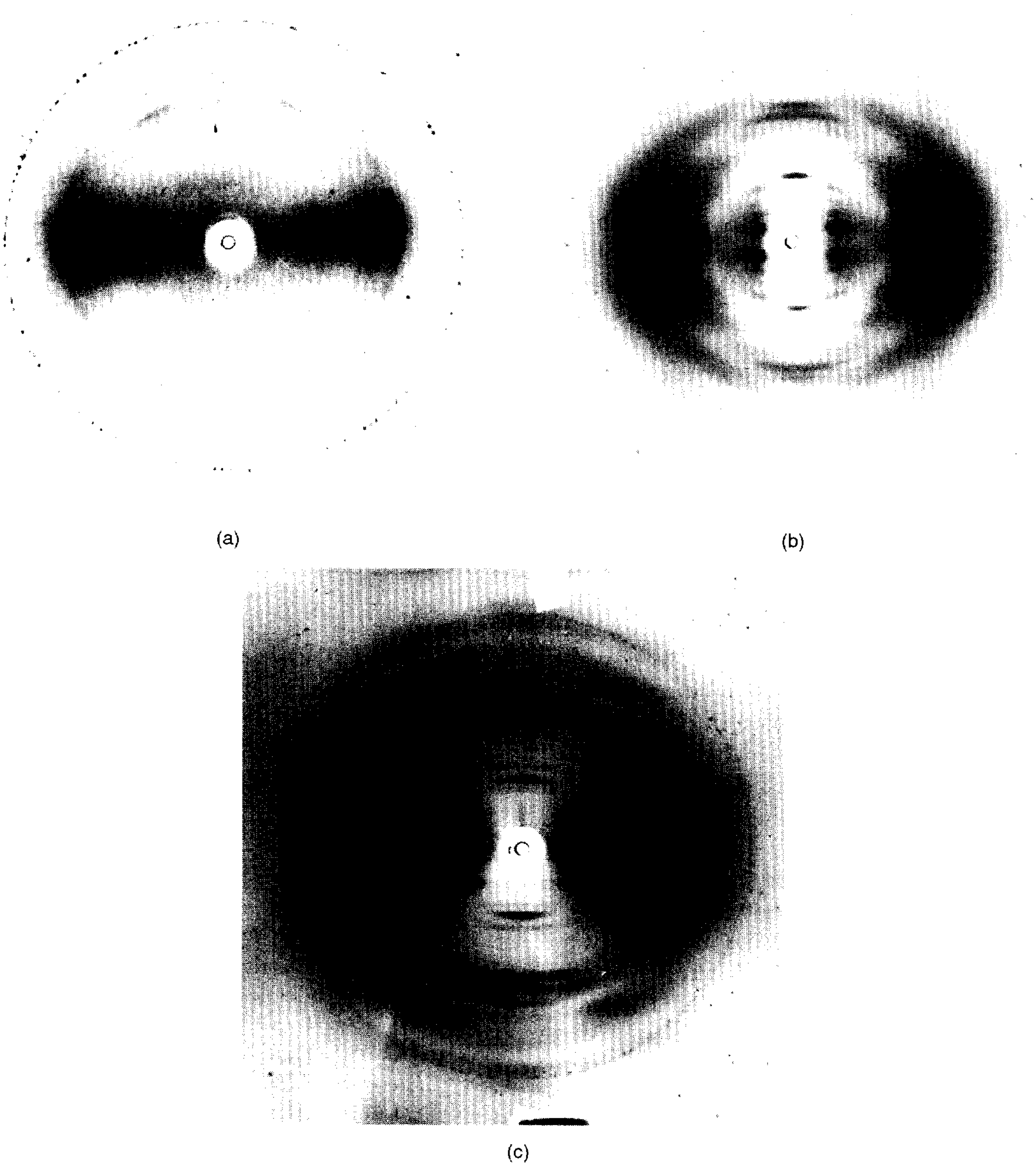

(a) (b) (c)

Fig. 4. X-ray fibre diffraction patterns for (a) native (acetylated) konjac mannan, (b) acetan, and (c) deacetylated acetan-konjac mannan (1:1) mixed gel. X-ray wavelength 0.154 nm. The samples were stretched 100% (konjac mannan), 100% (acetan), and 100% (deacetylated acetan–konjac mannan mixed gel).

Rheological studies have been made to confirm that the aqueous preparations gel so that any data obtained on the fibres is representative of structures present in gels. Clearly the conformation and interaction of acetan with other polysaccharides will be sensitive to the ionic environment and these aspects are discussed in more detail elsewhere [22,23]. At sufficiently high polymer concentrations the deacetylated acetan-konjac mannan mixtures formed transparent thermoreversible gels. Fig. 2 shows the mechanical spectrum for a 0.5% w/v 6:4 deacetylated acetan–konjac mannan gel. G' (storage modulus) > > G'' (loss modulus), and both are independent of frequency, indicative of true gels. Fig. 3 shows the dependence of G' on the composition of the mixture. The data shows synergistic gelation with the maximum G' value occurring at a deacetylated acetan–konjac mannan ratio of 6:4. Under the conditions for which the mixtures gel the individual components do not gel. This fact alone suggests intermolecular binding between the two polysaccharides, particularly in view of the evidence for binding obtained for xanthan–konjac mannan gels [4,9]. Fig. 4(a) shows a typical X-ray diffraction pattern obtained from a native 'acetylated' konjac mannan. The crystalline structure can be indexed onto the mannan II lattice (monoclinic: $a = 1.88$, $b = 1.87$, c (fibre axis) $= 1.02$ nm, $\gamma = 57.5$). The X-ray pattern for acetan [Fig. 4(b)] is consistent with the reported five-fold helical structure (pitch 4.8 nm). The X-ray fibre diffraction pattern for the deacetylated acetan–konjac mannan gel [Fig. 4(c)] provides direct evidence for intermolecular binding between the two polysaccharides. The pattern obtained is a new structure corresponding to the 'heterotypic' junction zones in the mixed gel. A simple mixture of the two polysaccharides would result in a pattern corresponding to one or either of the two polysaccharides or a superposition of the two patterns. Whereas xanthan-galactomannan mixed gels gave patterns related to the galactomannan crystal structure [2,3,9], the deacetylated xanthan–konjac mannan mixtures yielded patterns related to those of xanthan [4,9]. The deacetylated acetan–konjac mannan mixture X-ray diffraction pattern resembles that of acetan and xanthan. Within the limits of experimental accuracy the axial rise per chemical repeat unit appears to be the same (0.95 ± 0.01 nm) for acetan, xanthan, xanthan-konjac mannan and deacetylated acetan–konjac mannan mixed gels. However, whereas xanthan and acetan form five-fold helices the deacetylated acetan–konjac mannan mixture yields a pattern best characterised as a six-fold helix of pitch 5.6 ± 0.1 nm.The patterns obtained for deacetylated acetan–konjac mannan mixed gels are very similar to those obtained for xanthan–konjac mannan mixed gels [4,9]. Analysis of such patterns is complicated by the complex and possible irregular structure of the glucomannan. However the nature of the X-ray patterns, and the extreme sensitivity of the gelation process to the acetylation of the backbone [22,23], suggests that the junction zones of the gels could involve formation of novel mixed helical structures.

3. Conclusions

Direct evidence has been obtained for the intermolecular binding of deacetylated acetan and konjac mannan. The data suggests that the mechanisms for synergistic gelation of acetan or xanthan mixed gels are similar. Studies of mixed gels formed from 'xanthan-like' polysaccharides provide a basis for examining the effects of the length and composition of the sidechain, and the effects of non-carbohydrate substitution, on gelation. This is the fourth example of evidence for intermolecular binding between two polysaccharides, and the first example for a polysaccharide other than xanthan.

Acknowledgements

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